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(54) Title: METHOD OF DETECTING TISSUE-SPECIFIC FK506 BINDING PROTEIN MESSENGER RNAs AND USES THEREOF

(57) Abstract

A novel mRNA encoding FK506 binding protein (FKBP12) has been identified in human T lymphocytes. The cDNA corresponding to this mRNA has been sequenced and characterized. The mRNA has been shown to be identical in sequence in two regions to two previously known FKBP-encoding sequences and clearly distinct from the two known sequences in the 3' untranslated region (3'UTR). The tissue-distribution of this mRNA also differs from the tissue distribution of the two known mRNAs. DNA probes derived from this transcript encoding a distinct 3'UTR provide a means to detect FKBP12 mRNA in a tissue-specific manner and to use tissue-specific FKBP12 mRNA to monitor rejection of transplanted tissue, and the effects of FK506 immunosuppressant therapy. Nucleic acid sequences which hybridize to these 3'UTRs may also provide a means to modify *in vivo* production of tissue-specific FKBP mRNA.

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METHOD OF DETECTING TISSUE-SPECIFIC FK506 BINDING PROTEIN MESSENGER RNAs AND USES THEREOF

Background of the Invention

FK506 and cyclosporin A (CsA) are potent immunosuppressants that block the early steps of T lymphocyte activation. These immunosuppressants interfere with the Ca^{2+}-dependent signaling pathways mediated by the T cell receptor. (Tocci, M.J. et al., J. Immunol. 143:718-726 (1989); Mattila, P.S. et al., EMBO J. 9:4425-4433 (1990); Bierer, B.E. et al., Proc. Natl. Acad. Sci. USA 87:9231-9235 (1990). This common biological property is strikingly unpredictable from the distinct chemical structures of the two drugs, but can be rationalized by the recent finding that calcineurin, a Ca^{2+}- and calmodulin-dependent serine/threonine phosphatase (Klee, C.B. and Krinks, M.H. Biochemistry 17:120-126 (1978)), is a common target of these immunosuppressants when they are bound to specific intracellular receptors in vitro and in T lymphocytes. (Liu, J. et al., Cell 66:807-815 (1991); Friedman and Weissman, 1991). These receptors, termed immunophilins, apparently play key roles in immunosuppressive mechanisms and potentially offer insight into general cytoplasmic signaling and protein transport mechanisms within cells. (McKeon, F. Cell 66:823-826 (1991).

FKBPs (FK506-binding proteins) are members of a class of immunophilins that form complexes with FK506, as well as with the structurally related immunosuppressive macrolide rapamycin. (Schreiber, S.L. Science 251:283-287 1991). Both FK506 (Harding, M.W. et al., Nature 341:758-760 (1989; Siekierka, J.J. et al., Nature 341:755-757 (1989; Harrison, R.K. and Stein, R.L. Biochemistry 29:3813-3816 (1990) and rapamycin (Bierer B.E. et al., Proc. Natl. Acad. Sci. USA 87:9231-9235 (1990) inhibit the peptidyl-prolyl isomerase activity of FKBPs. The first FKBP to be described at the protein (Harding, M.W. et al., Nature 341:758-760 (1989; Siekerka, J.J. et al., Nature 341:755-757 (1989) and cDNA (Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990; Standaert, R. F. et al., Nature 346:671-674 (1990) levels was FKBP12, an abundant cytosolic immunophilin of M_r 11,800. More recently, FKBP13 (Jin, Y.J. et al., Proc. Natl. Acad. Sci. USA 88:6677-6681 (1991)) and FKBP25 (Galat, A., et al., Biochemistry 31:2427-2434 (1991) have been characterized as immunophilins that bind both FK506 and rapamycin. All of these FKBPs share a core consensus sequence with FKBP12 but only the FKBP12-FK506 complex inhibits calcineurin. This finding implies that the different FKBPs are likely to have diverse effects on signal transduction pathways even though their immunosuppressant binding properties reflect aspects of structural similarity.

The significant immunosuppressive activity of the FKBP12-FK506 complex makes it one of several drugs of choice for prophylactic therapy in transplant patients to prevent tissue rejection (Todo, S., et al., Ann Surg. 212:295-307 (1990); Fung, J. J. et al., Transplant. Proc. 23:2977-2983 (1991)).

Summary of the Invention

The present invention relates to the identification and characterization of a novel messenger RNA (mRNA) encoding an FK506 binding protein in human T lymphocytes and to the sequencing of its corresponding cDNA (SEQ ID NO:1). Sequencing of the cDNA reveals that the mRNA, also referred to as the RNA transcript, is identical in sequence in two regions (i.e., the 5' untranslated region (5'UTR) and the open reading frame region (ORF)) to two known FKBP12-encoding RNA sequences and clearly distinct from the two known sequences in the 3' untranslated region (3'UTR). Furthermore, the tissue distribution of the mRNA of the present invention, which is expressed predominantly in heart, placental and pancreatic tissue, differs from the tissue distribution of the other two mRNAs.

The present invention also relates to isolated DNA which is indicative of tissue-specificity FK506 binding protein mRNAs, and to DNA or RNA probes for use in the detection of nucleic acid sequences (mRNA and DNA) encoding FK506 binding protein mRNA present in specific tissue.

This invention further relates to a method of detecting the expression of tissue-specific FKBP12 mRNA as a means of monitoring tissue rejection in transplant patients. Thus far, indication of tissue rejection has been diagnosed by a combination of clinical, biochemical and pathological criteria. These present methods are complicated, time consuming, and still do not allow early detection. (Todo, S. et al. Ann. Surg. 212:295-307 (1990), Fung, J.J., et al. Transplant. Proc. 23:2977-2983 (1991)). For example, the diagnosis of liver allograft rejection is based on clinical manifestation of fever without a source of infection,

bil output or increased ascites; the biochemical indices of total bilirubin, SGOT, SGPT, alkaline phosphatase and other enzyme levels; and the pathological and histological evidence of tissue inflammation unique to tissue rejection situations.

In a preferred embodiment, RNA from tissue of a human is combined with a DNA or RNA probe which is complementary to all or a portion of the RNA sequence of the 3'UTR of a mRNA encoding FKBP12, the cDNA is shown in SEQ ID NO:2. Detection of hybridization is an indication of the presence of a mRNA encoding FKBP12 in specific tissue. The extent to which FKBP12 is produced can also be determined by western blot analysis of proteins purified from individual tissues. The present method is particularly advantageous because it makes it possible to detect tissue rejection due to an ineffective dosage of FK506 at an earlier stage than is possible with other available methods and, thus, to modify the drug dosage to increase the chances of tissue survival.

The present invention also relates to a method of determining the effects of FK506 therapy on an individual. Clinical management of immunosuppressive therapy treads a fine line between maintaining the optimal drug level which avoids rejection of the transplanted tissue, while minimizing the adverse effects associated with immunosuppressant drugs. Some of these adverse side effects include nephrotoxicity, malignant complications (e.g., posttransplant lymphoproliferative disease), alterations in glucose metabolism and neurotoxicity.

Therefore, it is critical to maintain stringent regulation of FK506 therapy, both in terms of over-all dosage and bioavailability to the specific tissues involved. RNA which is present in specific

tissue of a human can be hybridized to a DNA or RNA probe which is complementary to all or a portion of the RNA sequence of the 3'UTR of an FKBP12 mRNA, the cDNA is shown in SEQ ID NO:2. Detection of hybridization is indicative of mRNA present which encodes FKBP12, and in what amount. The amount of FKBP12 mRNA present is a reasonable indication of the amount of FKBP12 being produced in the target tissue. If insufficient FKBP12 is produced to be effective in immunosuppressive therapy, another line of therapy may be initiated before rejection or toxicity occurs.

The present invention also relates to a method of modifying production of FKBP12 in vivo. In the method, a synthetic oligonucleotide which hybridizes to all or a portion of the 3'UTR of an FKBP12 mRNA can be administered to an individual. The hybridization of the nucleotide sequence could result in modification of the production of FKBP12. In a preferred embodiment, the nucleotide sequence administered will be a DNA sequence, such that when it hybridizes to the 3'UTR of an FKBP12 mRNA translation will be modified.

Brief Description of the Drawings

Figure 1 shows the FKBP12A nucleic acid sequence. (SEQ ID NO: 1).

Figure 2 shows the FKBP12A 3' untranslated region (3'UTR) sequence. (SEQ ID NO: 2).

Figure 3 shows the comparison of the FKBPs, FKBP12A, FKBP12B and FKBP12C, including their respective 3'UTRs. (SEQ ID NOS: 1, 3 and 4 respectively).

Figure 4 shows th electrophoretic gel pattern of human genomic DNA digested with one of five

different restriction endonucleas s and then used for Southern analysis.

Figure 5 is a schematic representation of the human FKBP12 gene which spans approximately 17-30 kb and contains five exons that encode three different FKBP12 mRNAs.

Figure 6 is a schematic representation of three FKBP12 mRNAs (12A, 12B, 12C) encoded by the FKBP12 gene (SEQ ID NOS:14-25).

Figure 7A shows results of Northern analysis which indicates that poly(A)+RNA from E^+T cells enriched from human PBLs contains FKBP12A (0.9 kb), FKBP12B (1.7 kb), and FKBP12C (0.7kb) mRNA transcripts.

Figure 7B shows PCR analysis results of first-strand cDNA transcribed from cytoplasmic RNA and demonstrates that the FKBP12A and FKBP12B mRNAs are produced in an enriched, PHA-stimulated population of T lymphocytes.

Figure 8A shows poly(A)+RNA isolated from enriched populations of human E^+T cells that were stimulated for 0, 4, 16, or 48 hr with 10 $\mu g\ ml^{-1}$ PHA.

Figure 8B shows that a 72 hour exposure of the membrane used in 8A reveals that PHA stimulation also increases the abundance of the 0.9 kb FKBP12A mRNA transcripts.

Figure 9 depicts the electrophoretic gel pattern demonstrating that the 1.7 kb FKBP12 mRNA transcript is present in a variety of human tissues.

Figure 10 is a schematic representation showing the synthetic oligonucleotides and restriction enzyme cleavage sites used to generate probes specific to the different FKBP12 cDNAs and mRNA and the FKBP12 gene (SEQ ID NOS:8-11 and 26-33).

Detailed Description of the Invention

The present invention relates to the identification and characterization of a novel messenger RNA (mRNA) encoding an FK506 binding protein in human T lymphocytes and the sequencing of its corresponding cDNA. Sequencing of the corresponding cDNA revealed that the mRNA, or RNA transcript, is identical in sequence in two regions (i.e., the 5' untranslated region (5'UTR) and the open reading frame region (ORF)) to two known FKBP12-encoding RNA sequences and clearly distinct from the two known sequences in the 3' untranslated region (3'UTR). Furthermore, the tissue distribution of the mRNA of the present invention, which is expressed predominantly in heart, placental and pancreatic tissue, differs from the tissue distribution of the other two mRNAs.

The present invention more particularly relates to the cDNA clone containing an open reading frame that encodes a specific FK506 binding protein hereinafter referred to as FKBP12A (SEQ ID NO: 1), as shown in Figure 1.

As described in detail in Example 1, oligonucleotide probes specific to the FKBP12 open reading frame (ORF) sequence (Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990); Standaert, R.F. et al., Nature 346:671-674 (1990) were used to screen T cell cDNA libraries for other full-length FKBP12 cDNAs. Two different human T lymphocyte cDNA libraries yielded several candidate cDNA clones, and DNA sequence analysis indicated that one of the clones contained the complete FKBP12 ORF. Further analysis showed that the polyadenylated cDNA has a 5'UTR and ORF sequence identical to the cDNAs reported by Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990) and Standaert, R.F. et al., Nature 346:671-674 (1990),

and a 3'UTR distinct from those f the previously known FKBP12 cDNA clones (Figures 2 SEQ ID NO: 12, and 3 SEQ ID NOS: 1, 3 and 4). The newly cloned cDNA was designated FKBP12A (SEQ. ID NO: 1) and the others designated as FKBP12B (SEQ ID NO: 3) (Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990) and FKBP12C (SEQ ID NO: 4) (Standaert, R.F. et al., Nature 346:671-674 (1990). FKBP12A is 738 base pairs in length and is predicted to encode a protein of 108 amino acid residues (SEQ ID NO: 5). This protein sequence is identical to the deduced amino acid sequence of FKBP12 based on two published FKBP12 cDNA sequences. (Standaert, R.F., et al. Nature 346:671-674 (1990); Maki, N. et al., Proc. Natl. Acad. Sci. USA 87:5440-5443 (1990).

As described in detail in Example 2, Southern blot analysis of human genomic DNA was used to determine whether a single FKBP12 gene could encode the FKBP12 mRNAs predicted by the different cDNAs. Radiolabeled DNA fragments specific to the three FKBP12 cDNAs were used as hybridization probes against human genomic DNA cut to completion with one of five restriction endonucleases. Autoradiography revealed a single band in each fractionated digestion when a fragment specific to FKBP12A was used as the probe. (Figure 4, Lane 1 of each digest). Conversely, multiple bands were present when a sequence specific to FKBP12B and FKBP12C was used as the probe (Figure 4, Lane 2). A fragment containing the entire ORF of the cDNAs also produced these multiple bands (Figure 4, Lane 3), with variations only in the Hind III and Pst I digestions. Because the single bands visualized by the FKBP12A-specific probe were contained within each pattern generated by the other probes, it is reasonable to believe that the genomic sequences encoding the

different transcripts are linked. The simplest interpretation of these data suggests that a single FKBP12 gene encodes the different FKBP12 mRNAs and that two or more FKBP12 pseudogenes are specific to the ORF, FKBP12B, and FKBP12C sequences. This interpretation agrees with the report by DiLella, A.G. and Craig, R.J. Biochemistry 30:8512-8517 (1991) that the human FKBP12 gene has four related pseudogenes. As described in Example 3, the FKBP12 gene was then isolated and characterized. Its location was mapped to chromosome 20.

As described in Example 4, Northern hybridization and PCR analysis were used to identify the FKBP12 mRNAs transcribed from the FKBP12 gene. Hybridization analysis revealed that all three FKBP12 mRNAs are transcribed in erythrocyte receptor positive peripheral blood lymphocytes (E^+PBL's) (Figure 7A). The FKBP12A transcripts were best detected with a 3'UTR probe, presumably because they are less abundant than the FKBP12B and FKBP12C transcripts which compete for an ORF probe. Additionally, FKBP12B transcripts were made visible by autoradiography in half the time required to visualize the FKBP12C transcripts, presumably due to their relative abundance within the cells. These results suggest that the transcript abundance is FKBP12B>FKBP12C>FKBP12A. This interpretation is consistent with the finding that antisense RNA probes were required for efficient detection of the FKBP12A and FKBP12C mRNAs. The FKBP12A 3'UTR probe also hybridized to a 4.7 kb transcript population (Figure 7A, Lane 1) and, to a lesser degree, to two larger transcript populations (approximately 5.7 kd and 7.8 kb, visible in Figure 7A, Lane 2). These c uld represent cross-hybridizing mRNAs

unrelated to FKBP12 production or precursor populations of mature FKBP12 transcripts.

PCR analysis of first-strand cDNA demonstrated that cytoplasmic RNA of T lymphocytes stimulated with PHA (Figure 7B) contains FKBP12A and FKBP12B transcripts. The amplified FKBP12A sequences were consistently less abundant than the amplified FKBP12B sequences (Lane 2 and 3 in Figure 7B), corroborating the results of Northern analysis. Because FKBP12C is a subset of the FKBP12B sequence, internal primers could not distinguish the two transcript populations in PCR analysis.

Northern analysis also indicated that PHA stimulation of E^+T lymphocytes increased the cellular abundance of the FKBP12A and FKBP12B transcripts (Figure 8). The RNA isolated from enriched populations of human E^+T cells (5 μg per lane) was fractionated and probed with a synthetic DNA oligomer specific to 63 nucleotides of the FKBP12 ORF. A 16 hour exposure of the probed membrane reveals that PHA stimulation increases the abundance of the 1.7 kb FKBP12B transcripts. The first two time points examined after stimulation with 10 $\mu g\ ml^{-1}$ PHA, 4 hour and 16 hour, showed a progressive increase in these mRNA levels. The third time point examined, 48 hour, revealed an effect similar to that seen at 16 hour. It is reasonable to use standard laboratory techniques as described above, to determine whether the stimulatory effect could be detected prior to 4 hours and whether the effect was linearly or cyclically progressive. Additionally, hybridization experiments using antisense RNA probes rather than DNA probes would reasonably demonstrate whether FKBP12C transcripts also increase in abundance upon mitogenic stimulation.

Northern analysis demonstrated that the FKBP12 transcripts predominate in poly(A)+RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. As shown in Figure 9 each lane contained 2 μg of poly(A)+RNA from human tissue: H = heart, B = brain, Pl = placenta, Lu = lung, Li = liver, SM = skeletal muscle, K = kidney, Pa = pancreas. The radiolabeled probe was the DNA oligomer described in Figure 8. Positions of RNA molecular weight markers are shown in kb.

Two different probes both indicate the following approximate relative abundance of FKBP12B transcripts: heart=brain=placenta=lung>skeletal muscle=kidney>liver=pancreas. Heart, placental, and pancreatic tissue appear to contain the FKBP12A transcript (faint bands at 0.9 kb in Lanes H, Pl, and P of Figure 9). Heart and placental tissue also contained uncharacterized, faintly hybridizing RNAs around 4.2 kb.

The three FKBP12 transcripts are produced from a single gene that contains five exons (Figures 5 and 6 (SEQ ID NOS:14-25)) and is located on chromosome 20 (DiLella, A.G. and Craig R.J., Biochemistry 30:8512-8517 (1991)). The four intron-exon splice junctions contain the conserved elements characteristic of eukaryotic genes (Figure 5) and FKBP12 is encoded by the first four exons. The fifth exon provides a 3'UTR sequence exclusive to the FKBP12B and 12C transcripts. The four protein-coding exons correspond to the FKBP12 sequence as follows: Exon 1 encodes amino acids 1-11, Exon 2 encodes amino acids 13-27, Exon 3 encodes amino acids 29-65, and Exon 4 encodes amino acids 66-107 (arrows in Figure 3 designate exon junctions). Amino acids 12 and 28 are encoded by codons that are split by exon boundaries. There is a correlation between the

exon-encoded domains of FKBP12 and the structural motifs of the protein (Moore, J.M. et al., Nature 351:248-250 (1991) and DiLella, A.G. and Craig, R.J. Biochemistry 30:8512-8517 (1991)). Structural studies of uncomplexed FKBP12 (Michnick, S.W. et al., Science 252:836-839 (1991); Moore, J.M. et al., Nature 351:248-250 (1991) and the FKBP12/FK506 complex (Van Duyne, G.D. et al. Science 252:839-842 (1991) reveal a common hydrophobic pocket that is defined by residues of the five-stranded antiparallel ß-sheet on one face and by residues from a loop and single short helix on the opposite face. The residues that comprise the two central strands of the ß-sheet are encoded by Exon 4, and the amino acids that constitute the loop and helical region are encoded by Exon 3. X-ray crystallographic studies of the FKBP-FK506 complex reveal the hydrophobic pocket as the protein site that binds the immunosuppressant (Van Duyne,G.D. et al., Science 252:839-842 (1991), thus demonstrating that the residues encoded by Exons 3 and 4 also correspond to domains of function within FKBP12.

The unique 3'UTRs also suggest that stability, translatability, cellular location, and tissue specificity may affect FKBP12 production. (Jackson, R.J. and Standaert, N., Cell 62: 15-24 (1990); Lipshitz, H.D., Current Opinion in Cell Biology 3:966-975 (1991); Leff, S.E. and Rosenfeld, M.G., Ann. Rev. Biochem. 55:1091-1117 (1986)).

The general phenomenon of pseudogenes is well documented for a variety of eukaryotic genes. (Leff,S.E. et al., Ann. Rev. Biochem. 55:1091-1117 (1986). Poly(A) tails and 3'UTR sequences modulate mRNA half-life and translation efficiency in vivo (Jackson, R.J. and Standart, N. Cell 62:15-24 (1990). Therefore, transcript diversity generated by these

elements is reasonably viewed as a means to control the final production level of FKBP12 in cells. Additionally, 3'UTRs are known to regulate spatial expression of proteins by controlling proper transcript localization within cells (Lipshitz, H.D. Current Opinion in Cell Biology 3:966-975 (1991). It is reasonable to predict that regulated sequestration and translation of FKBP12 transcripts function to position FKBP12 near specific, localized cellular receptors to effect determined responses.

Finally, alternative 3'UTRs of otherwise identical messages also provide a means to control developmental- or tissue-specific expression of proteins (Leff, S.E. et al., Ann. Rev. Biochem. 55:1091-1117 (1986). It is also reasonable to predict that FKBP12A, FKBP12B and FKBP12C transcripts are regulated in a tissue-specific fashion, because the distribution of FKBP12B transcripts appears greater in heart, brain, placenta, and lung than in skeletal muscle, kidney, liver, and pancreas (Figure 9). This variance could affect the immunophilin:calcineurin ratio postulated to be important for FK506- and CsA-specificity.

Similarly, from the elevated accumulation of mature FKBP12A and FKBP12B transcripts in mitogenically stimulated T lymphocytes (Figure 8), it is reasonable to predict that one result of T cell activation could be increased demand for FKBP12 message, perhaps resulting in elevated FKBP12 levels. Of the three FKBP12 transcripts, the FKBP12A message is the one most likely to be tightly regulated in a tissue-specific fashion, since it does not given rise to the FKBP12 processed pseudogenes (see DiLella, A.G. and Craig, R.J. 1991).

The present invention also relates to isolated DNA, or RNA, having a nucleic acid sequence which is indicative of tissue-specificity of the mRNAs encoding FKBP12. At least three distinct messenger RNAs in human cells can encode FKBP12. Three of these transcripts encoding FKBP12A, FKBP12B and FKBP12C, have been characterized as described above and have been shown to differ only in their 3'UTRs (Figure 3) and their predominant expression in specific mammalian tissue. For example, FKBP12A is expressed in heart, placental and pancreatic tissues; FKBP12B, also found in these tissues, is additionally found in brain, lung, skeletal muscle, kidney and liver, in various quantities. Therefore, it is reasonable to predict that the 3'UTR provides a useful marker sequence to determine tissue-specificity of FKBP12 mRNAs.

The present invention also relates to probes which hybridize to all or a portion of the 3'UTR of FKBPs, and the use of these probes to detect the presence of tissue-specific FKBP12 mRNAs. For example, one type of DNA probe is comprised of a DNA sequence complementary to the 3'UTR of FKBP12A mRNA. Thus, hybridization of the FKBP12A DNA probe to RNA present in a tissue sample would be useful to detect FKBP12A mRNA present. Alternatively, an RNA probe could be used in a similar manner.

The present invention further relates to a method of monitoring rejection of transplanted tissue, or determining the effects of FK506 therapy to an individual. Production of FKBP12 is essential to form the inhibitory complex of FK506/FKBP12. The FK506/FKBP12 complex acts as a potent immunosuppressant. If insufficient FKBP12 is produced in the target tissue to be effective in immunosuppressive action, modifications of FK506

therapy may be made or another line of therapy may be initiated, before tissue rejection or systemic toxicity occurs.

The present invention also relates to a method of modifying production of FKBP12 in vivo. In the method, a nucleotide fragment, comprising either DNA or RNA complementary to DNA or RNA encoding FKBP12 is administered to an individual and hybridizes to all or a portion of the DNA or RNA encoding an FKBP. It is reasonable to predict that hybridization could result in modified production of the FKBP. For example, the nucleotide sequence administered can be a DNA sequence complementary to the 3'UTR of FKBP12A mRNA. When the sequence hybridizes to the 3'UTR, translation of the mRNA would be inhibited. Thus, production of FKBP12 in specific tissues such as heart, is decreased. A decreased production of heart FKBP12A could result in less competition for FK506 by heart tissue relative to, for example, kidney tissue. If a kidney had been the transplanted organ, the immunosuppressant activity could be concomitantly increased in that specific tissue. Thus, in vivo modification of tissue-specific FKBP12 could increase effective immunosuppressant therapy for targeted tissues.

The present invention further relates to in vivo and in vitro methods of evaluating the effectiveness of an agent as an inhibitor of FKBP12 mRNA production and, thus, usefulness in immunosuppressive therapy. Measurement of an agent as an inhibitor, may be accomplished by first, obtaining a baseline value of FKBP12 mRNA in cells a specific mammalian organ (i.e., the level of FKBP12 mRNA in the cells prior to or in the absence of treatment with an agent to be evaluated for its effectiveness as an immunosuppressant) using nucleic acid probes which

hybridize to all or a portion of the 3'UTR of FKBP12 mRNAs, as described above. The baseline value, is compared with the FKBP12 mRNA level in the same type of cells (e.g., a second sample or cells) after treatment with or in the presence of the agent being evaluated. Lower levels of FKBP12 mRNA after treatment with or in the presence of the agent (i.e., a post-treatment level of FKBP12 mRNA) is an indication that the agent inhibits FKBP12 mRNA production and, thus, can be used in immunosuppressive therapy. In an in vivo embodiment, the agent to be evaluated is administered to a mammal under conditions appropriate for the immunosuppressive agent to act upon production of FKBP12 mRNA. Subsequent to the administration of the agent, presence or absence or relative amounts of FKBP12 mRNA is determined using the nucleic acid probes of the method described. The difference between the baseline value and value obtained subsequent to administration of an agent shown to be an immunosuppressive agent, provides a reasonable basis of evaluating the effectiveness of the immunosuppressive agent in immunosuppressive therapy.

Alternately, cells may be isolated from mammalian tissue of interest, cultured in vitro, and a baseline value indicating the presence of FKBP12 mRNA in the cultured cells obtained as previously described. The cells are then treated with an agent to be evaluated as an immunosuppressant, under conditions appropriate for the agent to act upon production of FKBP12 mRNA in the cultured cells. As described, a lower FKBP12 mRNA level after treatment with or in the presence of an agent is an indication of its ability to inhibit FKBP12 mRNA production. Subsequent to th administration of the immunosuppressive agent, the presence or absence of FKBP12 mRNA is determined, with

the difference between the two values providing a reasonable basis of evaluating the effectiveness of the agent in immunosuppressive therapy.

The present invention will now be illustrated by the following examples, which are not to be seen as limiting in any way.

Example 1: Cloning and DNA Sequencing of FKBP-12A

Candidate FKBP12 cDNA clones were isolated from two human T lymphocyte cDNA libraries. A λgt10 cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) of peripheral blood T cells stimulated with phytohaemagglutinin (PHA) for 48 hr was screened under nonstringent conditions with two synthetic oligomers derived from the open reading frame (ORF) sequence of two FKBP12 cDNAs (Maki, N. et al., Proc.Natl. Acad. Sci. USA 87:5440-5443 (1990); Standaert, R.F. et al., Nature 346:671-674 (1990). The oligomers, (SEQ ID NOS: 6 and 7) were synthesized on an Applied Biosystems 380A DNA synthesizer and were radiolabeled with γ-^{32}P-ATP. A full-length FKBP12 cDNA obtained from this screen (FKBP12A) was used to screen a second stimulated T lymphocyte cDNA library for additional FKBP12 cDNAs. The genomic clone λVX18 was obtained by screening an EMBL-3 SP6/T7 library (Clontech) of human placental genomic DNA with ^{32}P-labeled FKBP12A cDNA under stringent conditions. Genomic clones cVX5 and cVX8 were obtained from a pWE15 cosmid library (Strategene Cloning Systems, La Jolla, CA) of human placental genomic DNA using hybridization probes under stringent conditions. cVX5 was isolated with a 4.5 kb fragment of λVX18 that extended from an Sfi I site just outside the SP6 promoter to the closest internal Kpn I site. cVX8 was isolated with a 764 bp fragment specific to the FKBP12B cDNA (generated by PCR using VX10230 and

VX10232 as primers as shown in Figure 10) and was analyzed further after demonstrating that it also hybridized to a 319 bp Ban II/EcoR I fragment specific to the FKBP12 cDNA. The 738 bp cDNA insert of FKBP12A was excised by EcoR I (all restriction enzymes obtained from New England Biolabs, Beverly, MA) digestion and cloned into pUC19 for sequence analysis. Portions of the genomic clones containing sequences homologous to the FKBP12 cDNAs were identified by hybridization and cloned into pUC19 or pGEM®-3Z (Promega, Madison, WI) for sequence analysis. All DNA sequences were determined using α-^{35}S-dATP (Biggin, M.D. et al., Proc. Natl. Acad. Sci. USA 80:3963-3965 (1983) and Sequenase® Version 2.0 DNA sequencing kits (USB, Cleveland, OH). Oligonucleotides were synthesized as required to serve as sequencing primers, and a commercial kit (USB) containing deoxy-7-deazaguanosine triphosphate was used to resolve compressions due to high G and C content (Mizusawa, S. et al., Nucleic Acid Res. 14:1319-1324 (1986). Both DNA strands were sequenced completely a minimum of two times, and all sequences were analyzed with computer software from DNAStar, Inc. (Madison, WI). The FKBP12A cDNA sequence (SEQ ID NO: 1) has been deposited in GenBank with accession number X52220. The intron and exon sequences of the human FKBP12 gene have been deposited in GenBank with accession numbers M80706, M92422, M92423, and M93060.

Example 2: Southern Analysis of Human Genomic DNA

Human genomic DNA (Clontech Laboratories, Inc. Palo Alto, CA) was digested with the restriction endonucleases BamH I, BsaB I, EcoR I, Hind III, or Pst I and fractionated (10 μg per lane) in 1% agarose. The DNA was transferred to GeneScreen (New England Nuclear Research Products, Boston, MA) by capillary transfer

using standard conditions and was crosslinked to the membrane with ultraviolet light (Stratalinker, Stratagene Cloning Systems, La Jolla, CA). The DNA probes were radiolabeled using α-^{32}P-dCTP and a commercial labeling kit (Pharmacia LKB Biotechnology, Piscataway, NJ) and were hybridized at 65°C under stringent conditions. The washed membranes were exposed at -70°C to X-Omat AR x-ray film (Eastman Kodak Company, Rochester, NY) using Cronex Lightning Plus (E.I. DuPont de Nemours and Company, Wilmington, DL) intensifying screens.

As shown in Figure 4, the hybridization probes were as follows: Lane 1 = 319 bp fragment specific to FKBP12A, generated by Ban II/EcoR I digestion of FKBP12A; Lane 2 = 524 bp fragment specific to FKBP12B and FKBP12C generated by PCR amplification of genomic DNA with primers VX10169 (SEQ ID NO:27) and VX10173 (SEQ ID NO:29); Lane 3 = 363 bp fragment, spanning the entire FKBP12 ORF, generated by Dde I digestion of FKBP12A DNA. DNA size markers are shown in kb and were determined by the migration profile of lambda DNA digested with EcoR I and Hind III.

Example 3: Characterization of the FKBP12 Gene and Chromosome Location

Using radiolabeled FKBP12A as a hybridization probe, a lambda library of human placental genomic/DNA was screened at high stringency. Four clones were selected and purified, and DNA from each clone was digested with restriction endonucleases and examined by Southern analysis. All clones hybridized to probes from the ORF, but they exhibited variable hybridization to probes from the 3' termini of the different FKBP12 cDNAs. One clone hybridized specifically to probes derived from th 3'UTRs of FKBP12B and FKBP12C, and sequence analysis indicated this clone was a processed

pseud gene. The three remaining clones hybridized specifically to probes from the 3'UTR of FKBP12A and were extremely similar by restriction enzyme analysis. One of these clones, λVX18, was analyzed further and proved to be a large portion of the FKBP12 gene.

Mapping studies showed that the λVX18 insert was approximately 15 kb in size and contained FKBP12A sequences localized at one end (Figure 5). Subcloning and sequence analysis revealed that the clone contained two exons (Exons 3 and 4) separated by a 3.2 kb intron and corresponding to the ultimate 616 bp of the FKBP12A cDNA. Exon 3 contained 113 bp of ORF while Exon 4 contained 126 bp of ORF and the 377 bp FKBP12A 3'UTR (Figure 6). Exon 3 was also contained within the FKBP12B and FKBP12C cDNA sequences, but only the first 165 bases of Exon 4 (the 126 bp of ORF, the TGA stop codon, and the next 36 bp) were found in these cDNA sequences (Figure 6). Additional library screens were performed to isolate genomic clones containing the 5' exons of the FKBP12 mRNAs and the 3' exons of the 12B and 12C mRNAs.

Next, a cosmid library with inserts averaging 38 kb in size was screened. Two independent screening approaches were used: (1) cosmid selection with a fragment from the 5' end of λVX18 and (2) cosmid selection with a DNA fragment from the 3'UTR of FKBP12B followed by secondary selection with a DNA fragment from the 3'UTR of FKBP12B followed by secondary selection of a clone containing 5' end of the FKBP12 gene; the second approach assumed linkage of the genomic sequences encoding the different FKBP12 cDNAs.

Clone cVX5 was isolated by the first approach and contained the 5' end of the FKBP12 gene; clone cVX8 was isolated by the second approach and contained the entire FKBP12 gene (Figure 5). Subcloning and

sequencing part of cVX5 revealed two exons (Exons 1 and 2) separated by an 80 bp intron. These exons encoded the 5'UTR and first 85 bp of ORF in each FKBP12 cDNA. Specifically, Exon 1 contained the upstream sequence and first 37 bp of ORF while Exon 2 encoded the next 48 bp of ORF (Figure 6). Subcloning and sequencing part of cVX8 identified Exon 5 as the exon encoding the 3' ends of the FKBP12B and 12C mRNAs. DNA sequence from cVX8 also demonstrated directly that the 3'UTR of the FKBP12A transcript lies within the intron between Exons 4 and 5 and is generated by read through of the 3' splice junction of Exon 4 (see Figures 5 and 6). In conjunction with mapping studies of cVX5 and λVX18, restriction and hybridization analysis of cVX8 indicated that the FKBP12 gene spans between 17-30 Kb.

Using PCR amplification to analyze DNA from human-hamster somatic cell lines representing the entire complement of human chromosomes, it was shown that amplification specific to the FKBP12 gene occurred only with DNA isolated from cell lines that contained human chromosome 20.

Human genomic DNA, genomic DNA from a Chinese hamster ovary cell line, and DNA from human-hamster hybrid somatic cell lines was obtained from Bios Corporation (New Haven, CT), and each was tested by PCR analysis for a sequence specific to the FKBP12 gene. The synthetic oligomers VX10027 and VX10247 (SEQ ID NOS: 8 and 9 respectively) served as the 5' and 3' primers, respectively. Each primer was used at a final concentration of 10 μM in conjunction with 50 ng of each cell line DNA, and the total reaction volume was 50 μl. Following 40 rounds of amplification (94°C, 1.5 min; 50°C, 1.5 min; 72°C, 3 min) on a MicroCycler E (Eppendorf, Fremont, CA), 10 μl of each reaction was fractionated by horizontal gel electroph resis in 3%

NuSieve agarose (FMC BioProducts) and visualized with ethidium bromide. The amplified DNA fragments were transferred and crosslinked to nylon membrane, and the membrane was probed with two ^{32}P-radiolabeled oligomers VX10171 and VX10174 (SEQ ID NOS: 10 and 11 respectively) internal to the amplified portion of the FKBP12 gene to confirm the identity of the amplified fragment. The amplified product was of the expected size (395 bp) and hybridized to synthetic DNA oligomers derived from the gene sequence and internal to the PCR primers.

Example 4: RNA Preparation, Northern Analysis and PCR Amplification from RNA

Fresh human peripheral blood lymphocytes (PBLs) were obtained from LeukoPaks (Children's Hospital Blood Bank, Boston, MA) derived from random, normal blood donors. The lymphocytes were isolated and separated by density centrifugation over Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) according to the manufacturer's instructions. The population was enriched for T lymphocytes by rosetting with sheep red blood cells for 1 hr at 4°C followed by another centrifugation through Histopaque-1077. Rosetted cells were recovered from the pellet after treatment with 0.16 M NH_4Cl, 0.01 M $KHCO_3$, 0.1 mM EDTA, pH 7.4. Erythrocyte receptor positive PBLS (E^+ PBLs) were either harvested immediately or were cultured at 37°C at a concentration of 10^7 cells ml^{-1} in RPMI 1640 (Gibco BRL, Grand Island, NY) containing 50 units ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, 2 mM L-glutamine, 50 μM ß-mercaptoethanol, 10% heat-inactivated fetal calf serum, 10 mM Hepes pH 7.3 (Gibco BRL), and 10 μg ml^{-1} PHA (Sigma) for 16 hr. All cells were then harvested at 250 x g for 10 min at room temperature and washed twic with Hank's balanced salt solution (Gibco

BRL). Total RNA was obtained by resuspending the washed cell pellet in 5-10 times its volume in 4.0 M guanidine thiocyanate, 0.1 M Tris-HCl pH 7.5, 1% ß-mercaptoethanol and extracting as described by Sambrook et al. (1989). Cytoplasmic RNA was purified as described by Gilman (1989). Poly(A)+RNA was selected with an mRNA spin column kit (5 Prime-3 Prime, Boulder, CO), resuspended in ribonuclease-free water, and stored at -70°C until use.

Poly(A)+RNA for Northern analysis was fractionated in 1% agarose containing formaldehyde before being transferred and crosslinked to nylon membrane as described above. The multiple tissue Northern blot was purchased from Clontech. DNA probes were radiolabeled with α-^{32}P-dCTP as described above while RNA probes were transcribed from fragments cloned into pBluescript® II (Stratagene Cloning Systems La Jolla, CA). The membranes were hybridized, washed, and exposed to x-ray film as described from Southern analysis. As shown in Figure 7A, Lane 1 = 20 hr exposure of the membrane; Lane 2 = 44 hr exposure. Marker sizes were determined by the migration profile of lambda DNA digested with EcoR I and Hind III and are indicated in kb.

RNA was prepared for polymerase chain reaction (PCR) analysis (Mullis, K.B. and Faloona, F.A. Methods Enzmol. 155:335-350 (1987); Saiki, R.K. et al., Science 239:487-491 (1988) using a modification of the procedure described by Brenner, C.A. et al. Bio/Techniques 7:1096-1103 (1989). Briefly, cytoplasmic RNA was primed with oligo-dT_{12-18} (Collaborative Research, Bedford, MA) and transcribed at 42°C for 1 hr with Moloney murine leukemia virus reverse transcriptase (BRL, Bethesda, MD) before being used for PCR reactions. DNA products visible by

ethidium bromide staining after thirty rounds of amplification were obtained using the first-strand cDNA produced from 0.17-1.3 μg of total cytoplasmic RNA. DNA oligomers used to amplify specific portions of the different FKBP12 mRNAs were as follows: VX10172 (SEQ ID NO:28)-VX10247 (SEQ ID NO:9) for FKBP12A mRNA, VX10027 (SEQ ID NO:8)-VX10173 (SEQ ID NO:29) for FKBP12B mRNA, and VX10172 (SEQ ID NO:28)-VX10028 (SEQ ID NO:26) for the ORF common to all FKBP mRNAs (see Figure 10). A Perkin Elmer Cetus GenAmp 9600 was used for all PCR reactions that involved cytoplasmic RNA, and the DNA products were fractionated on 2% NuSieve 3:1 (FMC BioProducts, Rockland, ME) agarose gels. As shown in Figure 7B, Lane 1 = VX10172 (SEQ ID NO:28)-VX10028 (SEQ ID NO:26): specific to the ORF of all FKBP12 mRNAs, 299 bp fragment expected and obtained; Lane 2 = VX10172 (SEQ ID NO:28)-VX10247 (SEQ ID NO:9): specific to FKBP12A mRNA, 683 bp fragment expected and obtained; Lane 3 = VX10027 (SEQ ID NO:28)-VX10173 (SEQ ID NO:29): specific to FKBP12B mRNA, 607 bp fragment expected and obtained; Lane 4 = VX10201 (SEQ ID NO:30)-VX10801 (SEQ ID NO:33): specific to FKBP12C mRNA, 608 bp fragment expected but not obtained; Lane 5 = control primers SEQ ID NOS: 12 and 13, respectively to produce a 335 bp fragment specific for ß2-microglobulin.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Vertex Pharmaceuticals, Inc.

(ii) TITLE OF INVENTION: METHOD OF DETECTING TISSUE-SPECIFIC FK506 BINDING PROTEIN MESSENGER RNAs AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 33

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(F) ZIP: 10020

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/886,611
(B) FILING DATE: 20-MAY-1992

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 27,794
(C) REFERENCE/DOCKET NUMBER: VPI92-01 (PCT)

(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 738 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGCCCGCCC GCTCAGCGTC CGCCGCCGCC ATGGAGTGC AGGTGGAAAC CATCTCCCCA 60

GGAGACGGGC GCACCTTCCC CAAGCGCGGC CAGACCTGCG TGGTGCACTA CACCGGGATG 120

CTTGAAGATG GAAAGAAATT TGATTCCTCC CGGGACAGAA ACAAGCCCTT TAAGTTTATG 180

CTAGGCAAGC AGGAGGTGAT CCGAGGCTGG GAAGAAGGGG TTGCCCAGAT GAGTGTGGGT 240

CAGAGAGCCA AACTGACTAT ATCTCCAGAT TATGCCTATG GTGCCACTGG GCACCCAGGC 300

ATCATCCCAC CACATGCCAC TCTCGTCTTC GATGTGGAGC TTCTAAAACT GGAATGACAG 360

GAATGGCCTC CTCCCTTAGC TCCCTGTTCT TGGGTAAGGA AATGGAATAC TGAAGGGCCC 420

TTCACTGCCT TTGCTCCTCC CATGTTATGC CCAGCGTTTG ATGGGTAGCA GAGAGAACAA 480

AAAACACCAC AAGGCTATTT TTCCCCCTGC ATTCTTTCTG TATTGAGTAT CCTTTCAGTG 540

TTATTAGTGT ATGCTTTGAA TGTAAAAATT GGTCACCCTA AGGAAAGGAA TTGGCATGTG 600

TATGTTCCCA GTTCAACTCA TGGAGATGGC AGCTGTTTAA ATGTTTTTCT ATGTAGTTTA 660

TAAATTAAAA CTGAATTGAG GACTATGGAA ATGTAGGCCA AATTTGTAGT GCCAACATTT 720

TAGTTCTTTG GAAAAAAA 738

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 374 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGGAATGGC CTCCTCCCTT AGCTCCCTGT TCTTGGGTAA GGAAATGGAA TACTGAAGGG 60

CCCTTCACTG CCTTTGCTCC TCCCATGTTA TGCCCAGCGT TTGATGGGTA GCAGAGAGAA 120

CAAAAAACAC CACAAGGCTA TTTTTCCCCC TGCATTCTTT CTGTATTGAG TATCCTTTCA 180

GTGTTATTAG TGTATGCTTT GAATGTAAAA ATTGGTCACC CTAAGGAAAG GAATTGGCAT 240

GTGTATGTTC CCAGTTCAAC TCATGGAGAT GGCAGCTGTT TAAATGTTTT TCTATGTAGT 300

TTATAAATTA AAACTGAATT GAGGACTATG GAAATGTAGG CCAAATTTGT AGTGCCAACA 360

TTTTAGTTCT TTGG 374

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1532 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCGGGC CGCCGCCAGG TGGCTGTTGG TCCACGCCGC CGGTCGCGCC GCCCGCCCGC 60

TCAGCGTCCG CCGCCGCCAT GGGAGTGCAG GTGGAAACCA TCTCCCCAGG AGACGGGCGC 120

ACCTTCCCCA AGCGCGGCCA GACCTGCGTG GTGCACTACA CCGGGATGCT TGAAGATGGA 180

AAGAAATTTG ATTCCTCCCG GGACAGAAAC AAGCCCTTTA AGTTTATGCT AGGCAAGCAG 240

GAGGTGATCC GAGGCTGGGA AGAAGGGGTT GCCCAGATGA GTGTGGGTCA GAGAGCCAAA 300

CTGACTATAT CTCCAGATTA TGCCTATGGT GCCACTGGGC ACCCAGGCAT CATCCCACCA 360

CATGCCACTC TCGTCTTCGA TGTGGAGCTT CTAAAACTGG AATGACAGGA ATGGCCTCCT 420

CCCTTAGCTC CCTGTTCTTG GATCTGCCAT GGAGGGATCT GGTGCCTCCA GACATGTGCA 480

CATGAGTCCA TATGGAGCTT TTCCTGATGT TCCACTCCAC TTTGTATAGA CATCTGCCCT 540

GACTGAATGT GTTCTGTCAC TCAGCTTTGC TTCCGACACC TCTGTTTCCT CTTCCCCTTT 600

CTCCTCGTAT GTGTGTTTAC CTAAACTATA TGCCATAAAC CTCAAGTTAT TCATTTTATT 660

TTGTTTTCAT TTTGGGGTGA AGATTCAGTT TCAGTCTTTT GGATATAGGT TTCCAATTAA 720

GTACATGGTC AAGTATTAAC AGCACAAGTG GTAGGTTAAC ATTAGAATAG GAATTGGTGT 780

TGGGGGGGGG GTTTGCAAGA ATATTTTATT TTAATTTTTT GGATGAAATT TTTATCTATT 840

ATATATTAAA CATTCTTGCT GCTGCGCTGC AAAGCCATAG CAGATTTGAG GCGCTGTTGA 900

GGACTGAATT ACTCTCCAAG TTGAGAGATG TCTTTGGGTT AAATTAAAAG CCCTACCTAA 960

AACTGAGGTG GGGATGGGGA GAGCCTTTGC CTCCACCATT CCCACCCACC CTCCCCTTAA 1020

ACCCCTCTGCC TTTGAAAGTA GATCATGTTC ACTGCAATGC TGGACACTAC AGGTATCTGT 1080

CCCTGGGCCA GCAGGGACCT CTGAAGCCTT CTTTGTGGCC TTTTTTTTTT TTCATCCTGT 1140

GGTTTTTCTA ATGGACTTTC AGGAATTTTG TAATCTCATA ACTTTCCAAG CTCCACCACT 1200

TCCTAAATCT TAAGAACTTT AATTGACAGT TTCAATTGAA GGTGCTGTTT GTAGACTTAA 1260

CACCCAGTGA AAGCCCAGCC ATCATGACAA ATCCTTGAAT GTTCTCTTAA GAAAATGATG 1320

CTGGTCATCG CAGCTTCAGC ATCTCCTGTT TTTTGATGCT TGGCTCCCTC TGCTGATCTC 1380

AGTTTCCTGG CTTTTCCTCC CTCAGCCCCT TCTCACCCCT TTGCTGTCCT GTGTAGTGAT 1440

TTGGTGAGAA ATCGTTGCTG CACCCTTCCC CCAGCACCAT TTATGAGTCT CAAGTTTTAT 1500

TATTGCAATA AAAGTGCTTT ATGCCCGAAT TC 1532

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 583 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCGCCGCCAT GGGAGTGCAG GTGGAAACCA TCTCCCCAGG AGACGGGCGC ACCTTCCCCA 60

AGCGCGGCCA GACCTGCGTG GTGCACTACA CCGGGATGCT TGAAGATGGA AAGAAATTTG 120

ATTCCTCCCG GGACAGAAAC AAGCCCTTTA AGTTTATGCT AGGCAAGCAG GAGGTGATCC 180

GAGGCTGGGA AGAAGGGGTT GCCCAGATGA GTGTGGGTCA GAGAGCCAAA CTGACTATAT 240

CTCCAGATTA TGCCTATGGT GCCACTGGGC ACCCAGGCAT CATCCCACCA CATGCCACTC 300

TCGTCTTCGA TGTGGAGCTT CTAAAACTGG AATGACAGGA ATGGCCTCCT CCCTTAGCTC 360

CCTGTTCTTG GATCTGCCAT GGAGGGATCT GGTGCCTCCA GACATGTGCA CATGAGTCCA 420

TATGGAGCTT TTCCTGATGT TCCACTCCAC TTTGTATAGA CATCTGCCCT GACTGAATGT 480

GTTCTGTCAC TCAGCTTTGC TTCCGACACC TCTGTTTCCT CTTCCCCTTT CTCCTCGTAT 540

GTGTGTTTAC CTAAACTATA TGCCATAAAC CTCAAGTTAT TCA 583

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gly Val Gln Val Glu Thr Ile Ser Pro Gly Asp Gly Arg Thr Phe
1 5 10 15

Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr Thr Gly Met Leu Glu
20 25 30

Asp Gly Lys Lys Phe Asp Ser Ser Arg Asp Arg Asn Lys Pro Phe Lys
35 40 45

Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly Trp Glu Glu Gly Val
50 55 60

Ala Gln Met Ser Val Gly Gln Arg Ala Lys Leu Thr Ile Ser Pro Asp
65 70 75 80

Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile Ile Pro Pro His Ala
85 90 95

Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu Glu
100 105

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AACAAGCCCT TTAAGTTTAT G 21

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATAAACTTA AAGGGCTTGT T 21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTGGTCTTTG ATGTGGAGC 19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTTGGCACTA CAAATTTGGC CTAC 24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCAATTCCT TTCCTTAGGG 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCCTTCACT GCCTTTGCTC 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTGGGGCTAC TCTCTCTTTC TGG 23

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCTTACATGT CTGATCCCAC TTAA 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGACGGUGA GUAG 14

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCCCGUCUCU GUCCCCUCAG 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGCGCA 6

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ACACGGUGA GUCG 14

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

UACAGUGGUC UUUUUCACAG 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGAUGC 6

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCCCAGGUAU GCUC 14

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CUCCUUGUU CUUUCACAG 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AUGAGU 6

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

UCUUGGGUAA GGAA 14

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AGADDDDDDG DCCCCAACAG 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ADDDGC 6

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CATGGAAGAC GAGAGTGGC 19

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GAGGGATCTG GTGCCTCCAG 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAGTGCAGGT GGAAACCATC 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CATCCCCACC TCAGTTTAG 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCTCAGGGTC CGCCGCCGCC 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGGGTGAAGA TTCAGTTTCA G 21

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CACTACACAG GACAGCAAAG G 21

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTTTTTTTTT TTTTGAATA ACTTGAGG 28

CLAIMS

1. A cDNA clone of mammalian origin encoding an FK506 binding protein which is expressed predominantly in heart tissue, placental tissue and pancreatic tissue.

2. The cDNA clone of claim 1, said clone being of human origin.

3. The cDNA clone of claim 1, said clone comprising all or a portion of the nucleic acid sequence of SEQ ID NO: 1.

4. The cDNA clone of claim 3, said clone consisting essentially of the nucleic acid sequence of SEQ ID NO: 1.

5. An isolated DNA of mammalian origin, said DNA comprising all or a portion of the nucleic acid sequence of SEQ ID NO: 1.

6. The isolated DNA of claim 5, said DNA consisting essentially of the nucleic acid sequence of SEQ ID NO: 1.

7. An isolated DNA which encodes FKBP12 and comprises an untranslated 3'UTR nucleic acid sequence, said sequence being indicative of tissue distribution of FKBP12 mRNA.

8. The isolated DNA of claim 7, said untranslated 3' UTR nucleic acid sequence indicating expression of FDKP12 in RNA by a specific tissue.

9. An isolated DNA, said DNA comprising all or a portion of the nucleotide sequence of SEQ ID NO: 2.

10. The isolated DNA of claim 9, said DNA consisting essentially of the nucleotide sequence of SEQ ID NO: 2.

11. An isolated DNA of mammalian origin which hybridizes to a DNA or RNA fragment having a nucleic acid sequence indicative of tissue specific production of FKBP12 mRNAs.

12. The isolated DNA of claim 11, wherein said DNA hybridizes to a DNA or RNA fragment and comprises an untranslated 3' UTR nucleic acid sequence indicating expression of FKBP12 mRNA by a specific tissue.

13. The isolated DNA of claim 11, said DNA being of human origin.

14. The isolated DNA of claim 11, wherein said DNA hybridizes to all or a portion of the nucleotide sequence of SEQ ID NO: 2.

15. A method of detecting FKBP12 mRNAs expressed in a mammalian tissue, comprising the steps of:

a) obtaining a sample of mammalian tissue;

b) preparing the sample of mammalian tissue so as to render RNA present therein available for hybridization with complementary nucleic acid, th reby producing a treated sample;

c) contacting the treated sample with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNAs; and

d) detecting hybridization of the nucleic acid probe with RNA from the treated sample, wherein hybridization is an indication of the presence of FKBP12 mRNA.

16. The method of claim 15, wherein the nucleic acid probe hybridizes to all or part of the nucleotide sequence of SEQ ID NO: 1.

17. The method of claim 15, wherein the nucleic acid probe hybridizes to all or a part of the nucleotide sequence of SEQ ID NO: 2.

18. A method of monitoring rejection of transplanted mammalian tissue, comprising the steps of:

a) obtaining a sample of transplanted tissue;

b) preparing the sample of transplanted tissue so as to render RNA present in the sample available for hybridization with complementary nucleic acid, thereby producing a treated sample;

c) contacting the treated sample with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNA; and

d) detecting hybridization of the nucleic acid probe with RNA from the treated sample, wherein hybridization is an indication of the presence of FKBP12 mRNAs.

19. A method of monit ring the effects of FK506 therapy in a mammal comprising the steps of:

a) obtaining a sample of mammalian tissue;

b) preparing the sample of mammalian tissue so as to render RNA present therein available for hybridization with complementary nucleic acid, thereby producing a treated sample;

c) contacting the treated sample with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNA; and

d) detecting hybridization of the probe with RNA from the treated sample, wherein the extent of hybridization is indicative of the extent to which FKBP12 mRNA is present in the treated sample and hybridization below a threshold level is indicative of insufficient FKBP12 mRNA level in the tissue.

20. A method of modifying production of FKBP in an individual, comprising administering to the individual a nucleic acid sequence which hybridizes to all or a portion of the 3'UTR of FKBP12 mRNA such that hybridization results in the modified production of FKBP12.

21. The method of claim 15, wherein the nucleic acid sequence is a DNA or RNA sequence which hybridizes to all or a portion of the nucleotide sequence of SEQ ID NO: 1.

22. An <u>in vivo</u> method of evaluating the effectiveness of an agent as an inhibitor of FKBP12 mRNA production in mammalian cells, comprising the steps of:

a) obtaining a first sample of cells from a mammalian tissue of interest;

b) preparing the first sample of cells so as to render RNA present in the sample available for hybridization with complementary nucleic acid, thereby producing a treated sample;

c) contacting the first sample of treated cells with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNAs;

d) detecting hybridization of the nucleic acid probe with FKBP12 mRNA from the first sample of treated cells, thereby obtaining a baseline value of FKBP12 mRNA;

e) administering to the mammal the agent to be evaluated, under conditions appropriate for said agent to act upon mRNA production;

f) obtaining a second sample of cells from the mammalian tissue sampled in a);

g) preparing the second sample of treated cells so as to render RNA present therein available for hybridization with complementary nucleic acid, thereby producing a second sample of treated cells;

h) contacting the second sample of treated cells with a nucleic acid probe which hybridizes to the 3' UTR of FKBP12 mRNAS; and

i) detecting hybridization of the nucleic acid probe with FKBP12 mRNA from the second sample of treated cells, thereby obtaining a post-treatment level of FKBP12 mRNA, wherein a lower post-treatment level of FKBP12 mRNA than baseline value is an indication that the agent inhibits FKBP12 mRNA thereby identifying an inhibitor of FKBP12 mRNA production.

23. An <u>in vitro</u> method of valuating the effectiveness of an agent as an inhibitor of FKBP12

mRNA production in mammalian cells, comprising the steps of:

a) obtaining cells from mammalian tissue of interest and culturing said mammalian cells under conditions appropriate for growth;

b) obtaining a first sample of cells from the cultured cells of step a);

c) preparing said first sample of cells so as to render RNA present in the cells available for hybridization with complementary nucleic acid, thereby producing a first sample of treated cells;

d) contacting the first sample of treated cells with a nucleic acid probe which hybridizes to the 3'UTR of FKBP12 mRNAs;

e) detecting hybridization of the nucleic acid probe with FKBP12 mRNA from the first sample of treated cells, thereby obtaining a baseline value of FKBP12 mRNA;

f) treating said cultured mammalian cells of step a) with the agent to be evaluated, under conditions appropriate for said agent to act upon mRNA production;

g) obtaining a second sample of cells from the cultured mammalian cells of step f);

h) preparing the second sample of cells so as to render RNA present therein available for hybridization with complementary nucleic acid, thereby producing a second sample of treated cells;

i) contacting the second sample of treated cells with a nucleic acid probe which hybridizes to the 3' UTR of FKBP12 mRNAS; and

j) detecting hybridization of the nucleic acid probe with FKBP12 mRNA from the second sample of treated cells, thereby obtaining a post-

treatment level of FKBP12 mRNA, wherein a lower post-treatment level of FKBP12 mRNA than baseline value is an indication that the agent FKBP12 inhibits mRNA production, thereby identifying an inhibitor of FKBP12 mRNA production.

FIGURE 1

CCGCCCGCCCGCTCAGCGTCCGCCGCCGCCATGGGAGTGCAGGTGGAAACCATCTCCCCAG
GAGACGGGCGCACCTTCCCCAAGCGCGGCCAGACCTGCGTGGTGCACTACACCGGGATGCT
TGAAGATGGAAAGAAATTTGATTCCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATGCTA
GGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCCAGATGAGTGTGGGTCAGA
GAGCCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCAGGCATCAT
CCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACTGGAATGACAGGAATGG
CCTCCTCCCTTAGCTCCCTGTTCTTGGGTAAGGAAATGGAATACTGAAGGGCCCTTCACTG
CCTTTGCTCCTCCCATGTTATGCCCAGCGTTTGATGGGTAGCAGAGAGAACAAAAAACACC
ACAAGGCTATTTTTCCCCCTGCATTCTTTCTGTATTGAGTATCCTTTCAGTGTTATTAGTG
TATGCTTTGAATGTAAAAATTGGTCACCCTAAGGAAAGGAATTGGCATGTGTATGTTCCCA
GTTCAACTCATGGAGATGGCAGCTGTTTAAATGTTTTTCTATGTAGTTTATAAATTAAAAC
TGAATTGAGGACTATGGAAATGTAGGCCAAATTTGTAGTGCCAACATTTTAGTTCTTTGGA
AAAAAA

FIGURE 2

CAGGAATGGCCTCCTCCCTTAGCTCCCTGTTCTTGGGTAAGGAAATGGAATACTGAAGGGCCCTT
CACTGCCTTTGCTCCTCCCATGTTATGCCCAGCGTTTGATGGGTAGCAGAGAGAACAAAAAACAC
CACAAGGCTATTTTTCCCCCTGCATTCTTTCTGTATTGAGTATCCTTTCAGTGTTATTAGTGTAT
GCTTTGAATGTAAAAATTGGTCACCCTAAGGAAAGGAATTGGCATGTGTATGTTCCCAGTTCAAC
TCATGGAGATGGCAGCTGTTTAAATGTTTTTCTATGTAGTTTATAAATTAAAACTGAATTGAGGA
CTATGGAAATGTAGGCCAAATTTGTAGTGCCAACATTTTAGTTCTTTGG

FIGURE 3

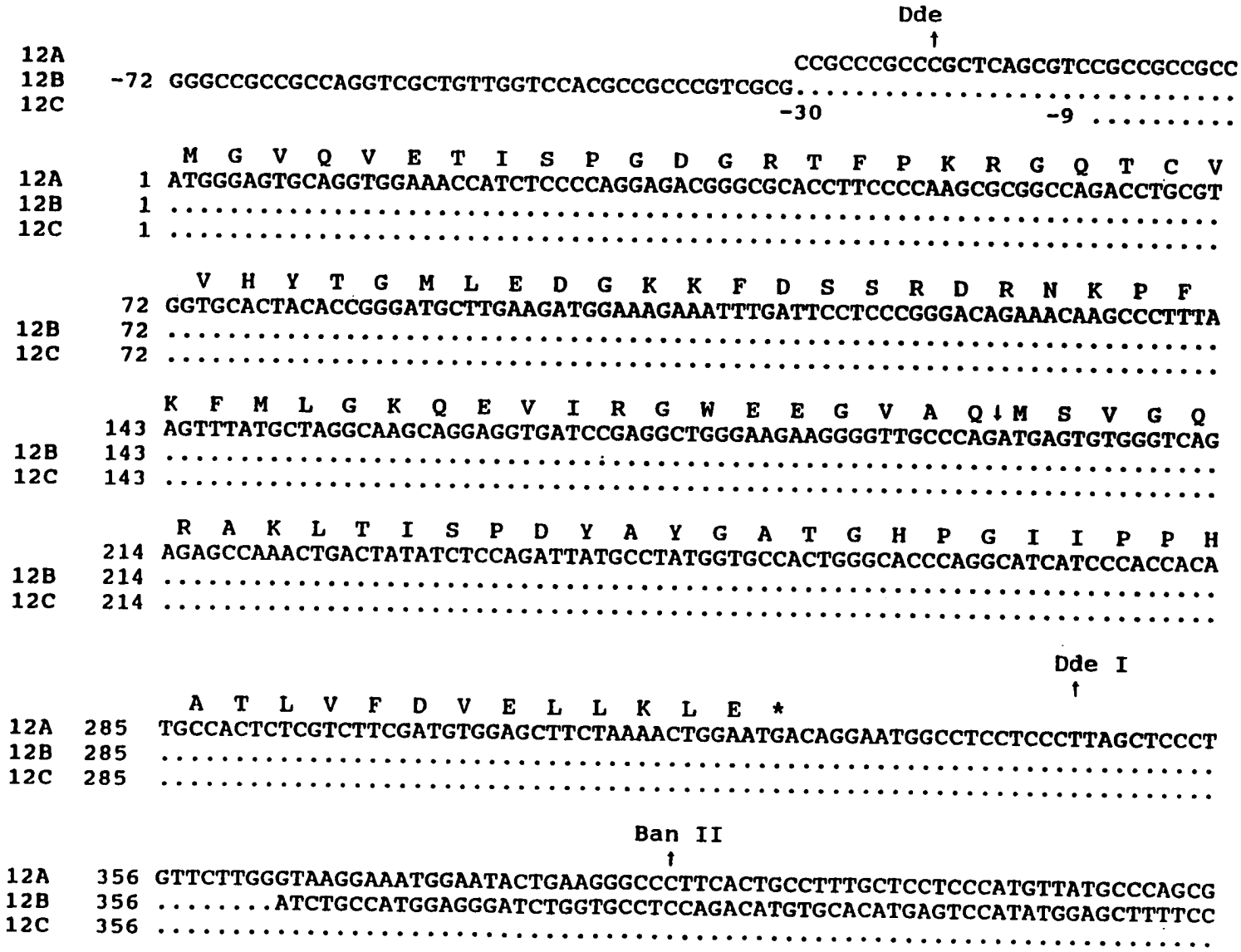

FIGURE 3 (continued)

```
12A    427 TTTGATGGGTAGCAGAGAGAACAAAAAACACCACAAGGCTATTTTTCCCCCTGCATTCTTTCTGTATTGAG
12B    427 TGATGTTCCACTCCACTTTGTATAGACATCTGCCCTGACTGAATGTGTTCTGTCACTCAGCTTTGCTTCCG
12C    427 ............................................................

12A    498 TATCCTTTCAGTGTTATTAGTGTATGCTTTGAATGTAAAAATTGGTCACCCTAAGGAAAGGAATTGGCATG
12B    498 ACACCTCTGTTTCCTCTTCCCCTTTCTCCTCGTATGTGTGTTTACCTAAACTATATGCCATAAACCTCAAG
12C    498 ..........................................................CATAAA.......

12A    569 TGTATGTTCCCAGTTCAACTCATGGAGATGGCAGCTGTTTAAATGTTTTTCTATGTAGTTTATAAATTAAA
12B    569 TTATTCATTTTATTTTGTTTTCATTTTGGGGTGAAGATTCAGTTTCAGTCTTTTGGATATAGGTTTCCAAT
12C    569 ......A_n

12A    640 ACTGAATTGAGGACTATGGAAATGTAGGCCAAATTTGTAGTGCCAACATTTTAGTTCTTTGGA_n
12B    640 TAAGTACATGGTCAAGTATTAACAGCACAAGTGGTAGGTTAACATTAGAATAGGAATTGGTGTTGGGGGGG

12B    711 GGGTTTGCAAGAATATTTTATTTTAATTTTTTGGATGAAATTTTTATCTATTATATATTAAACATTCTTGC

12B    782 TGCTGCGCTGCAAAGCCATAGCAGATTTGAGGCGCTGTTGAGGACTGAATTACTCTCCAAGTTGAGAGATG

12B    853 TCTTTGGGTTAAATTAAAAGCCCTACCTAAAACTGAGGTGGGGATGGGGAGAGCCTTTGCCTCCACCATTC

12B    924 CCACCCACCCTCCCCTTAAACCCTCTGCCTTTGAAAGTAGATCATGTTCACTGCAATGCTGGACACTACAG

12B    995 GTATCTGTCCCTGGGCCAGCAGGGACCTCTGAAGCCTTCTTTGTGGCCTTTTTTTTTTTTCATCCTGTGGT

12B   1066 TTTTCTAATGGACTTTCAGGAATTTTGTAATCTCATAACTTTCCAAGCTCCACCACTTCCTAAATCTTAAG

12B   1137 AACTTTAATTGACAGTTTCAATTGAAGGTGCTGTTTGTAGACTTAACACCCAGTGAAAGCCCAGCCATCAT

12B   1208 GACAAATCCTTGAATGTTCTCTTAAGAAAATGATGCTGGTCATCGCAGCTTCAGCATCTCCTGTTTTTTGA

12B   1279 TGCTTGGCTCCCTCTGCTGATCTCAGTTTCCTGGCTTTTCCTCCCTCAGCCCCTTCTCACCCCTTTGCTGT

12B   1350 CCTGTGTAGTGATTTGGTGAGAAATCGTTGCTGCACCCTTCCCCCAGCACCATTTATGAGTCTCAAGTTTT

12B   1421 ATTATTGCAATAAAAGTGCTTTATGCCCGGCTTTTCTCA_n
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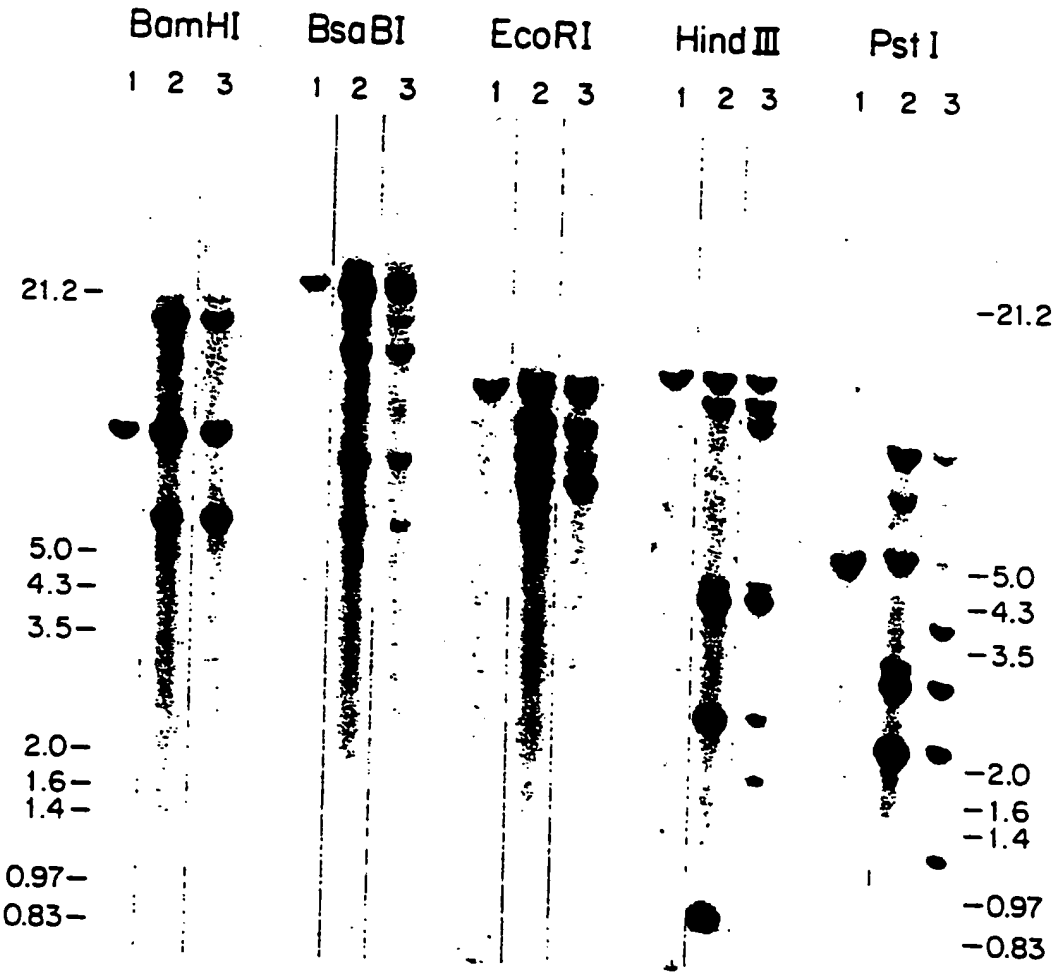

BamHI
BsaBI
EcoRI
Hind III
Pst I
1 2 3
21.2–
5.0–
4.3–
3.5–
2.0–
1.6–
1.4–
0.97–
0.83–
–21.2
–5.0
–4.3
–3.5
–2.0
–1.6
–1.4
–0.97
–0.83

FIG. 4

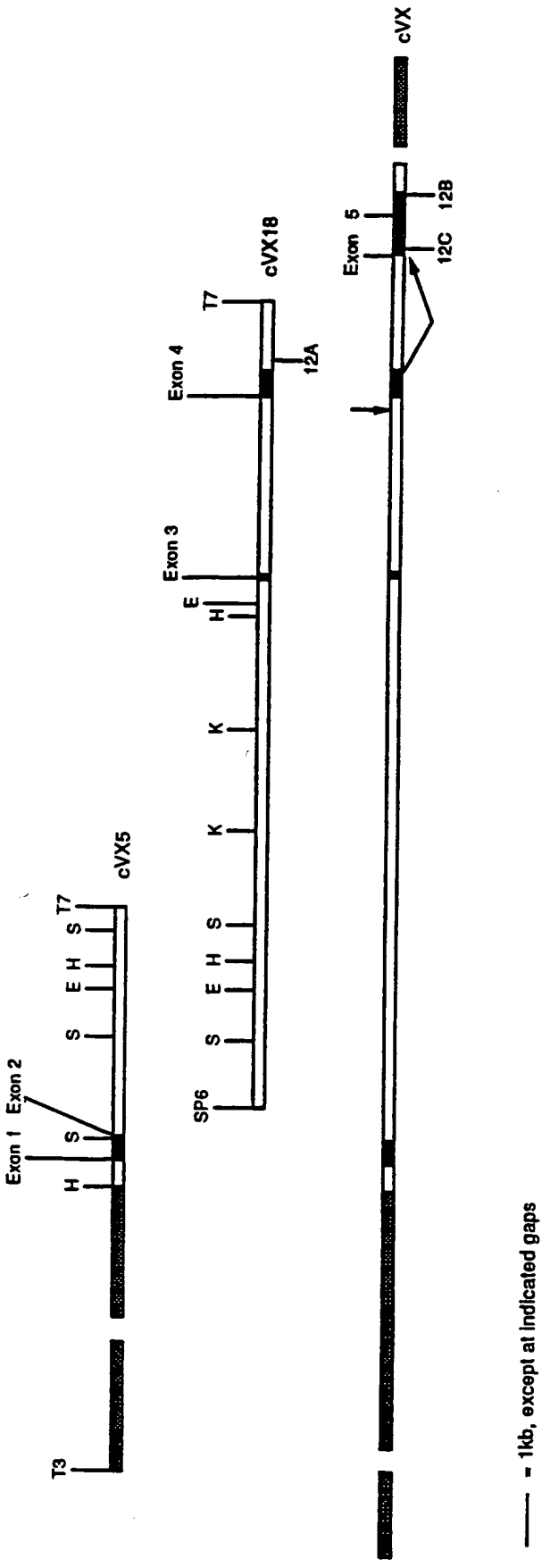
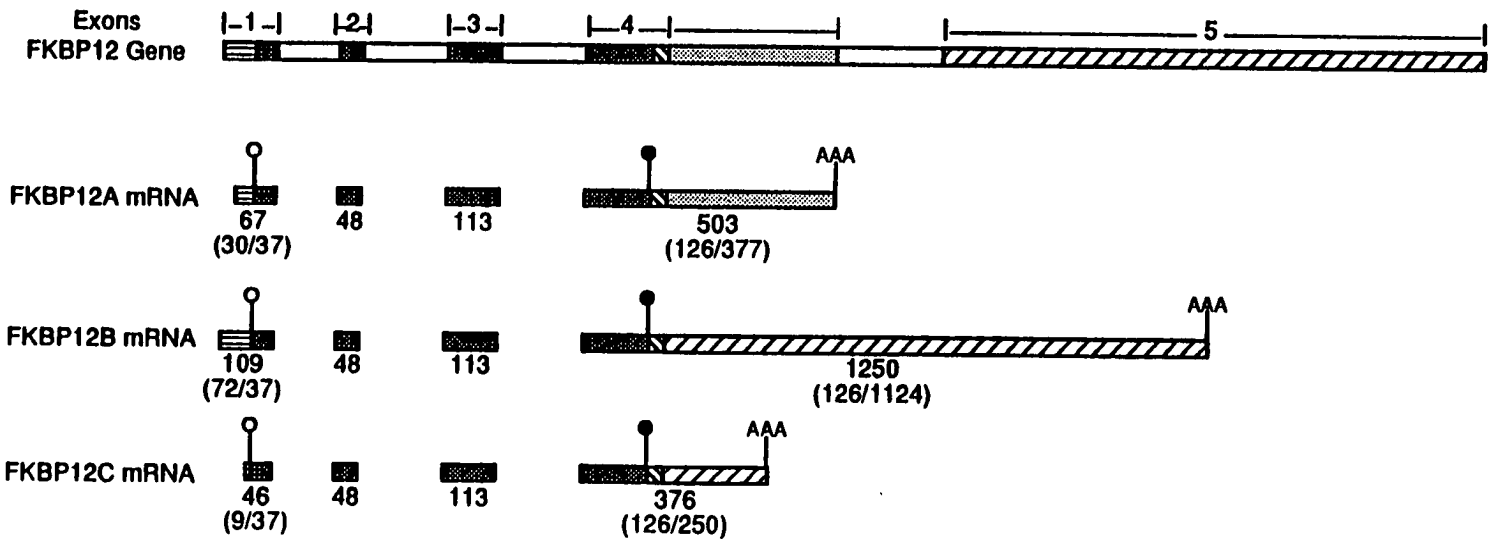

cVX
Exon 5
12B
12C
cVX18
T7
Exon 4
12A
Exon 3
E
H
K
K
S
E
H
S
SP6
cVX5
T7
S
E
H
S
Exon 2
Exon 1
S
H
T3
= 1kb, except at indicated gaps

FIGURE 5

FIGURE 6



= 5' UTR of Exon 1
= ORF sequence
= Intron sequence
= 36 bp common element
= Intron sequence retained as exon sequence in FKBP12A mRNA
= Exon 5 used only in FKBP12B & FKBP12A mRNAs
O = ATG start
=TGA stop
AAA = poly(A) tail

	EXON	INTRON	EXON
Splice junction 1/2	GAGACG	GUGAGUAG...GCCCGUCUCUGUCUCCUCAG (80 bp)	GGCGCA
Splice junction 2/3	ACACCG	GUGAGUCG...UACAGUGGUCUUUUUCACAG (~10 kb)	GGAUGC
Splice junction 3/4	GCCCAG	GUAUGCUC...CUUCCUUGUUCUUUUCACAG (3.2 kb)	AUGAGU
Splice junction 4/5	UCUUGG	GUAAGGAA...AGAUUUUCUGUCCCCAACAG (~2.2 kb)	ATCTGC

FIG. 7A

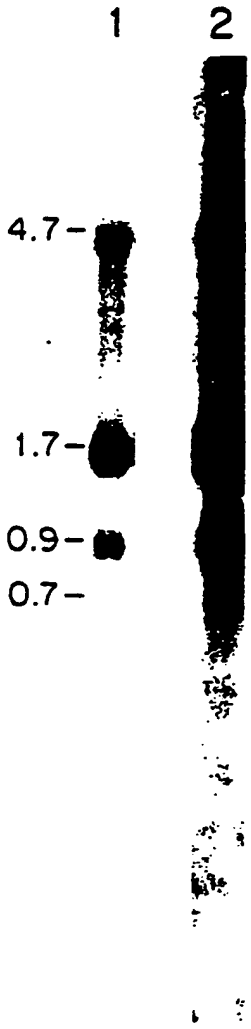
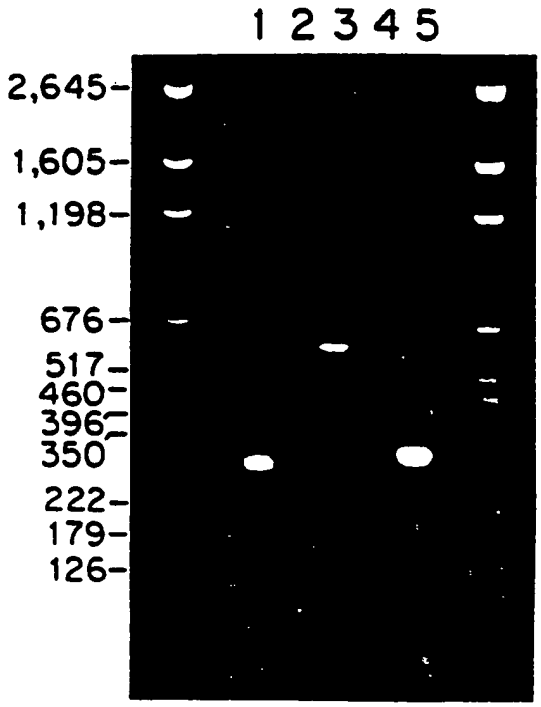

1
2
4.7
1.7
0.9
0.7

FIG. 7B


1 2 3 4 5
2,645
1,605
1,198
676
517
460
396
350
222
179
126

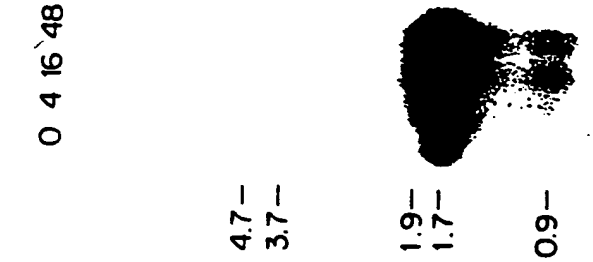


FIG. 8B

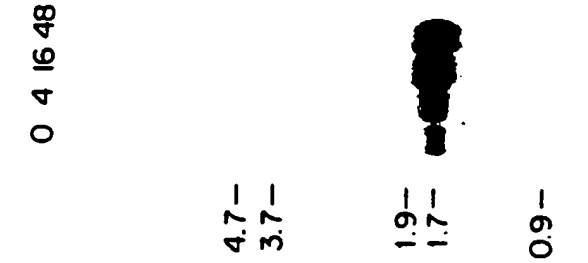


FIG. 8A

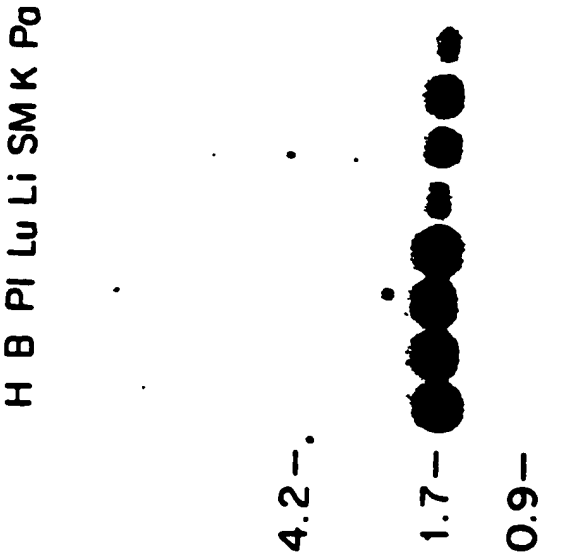
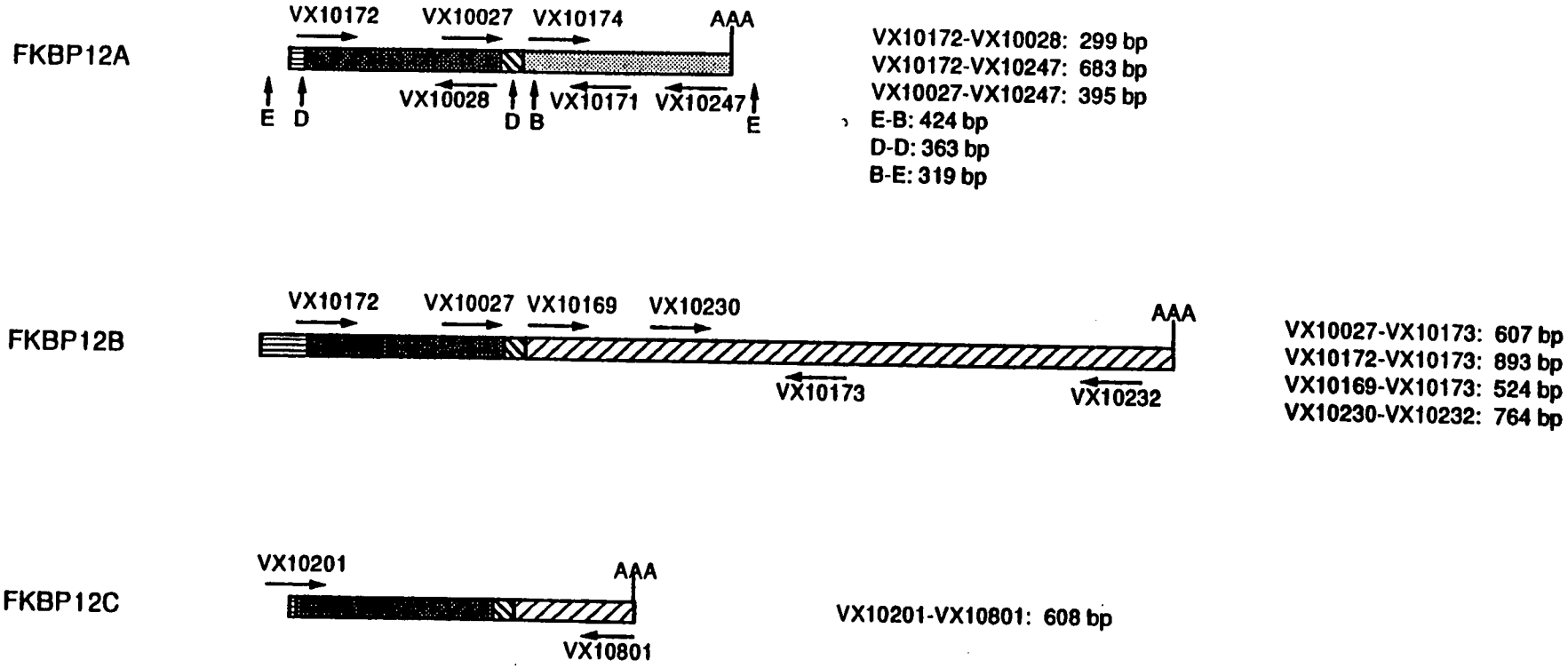


FIG. 9

FIGURE 10



Synthetic oligonucleotide sequences

VX10027: CTCGTCTTCGATGTGAGC
VX10169: GAGGGATCTGGTCCTCCAG
VX10171: GCCAATTCCTTTCCTTAGGG
VX10172: GAGTGCAGGTGGAAACCATC
VX10173: CATCCCCACCTCAGTTTTAG
VX10174: GCCCTTCACTGCCTTTGCTC
VX10201: GCTCAGCGTCCGCCGCCGCC
VX10230: GGGGTGAAGATTCAGTTTCAG
VX10232: CACTACACAGGACAGCAAAGG
VX10247: GTTGGCACTACAAATTTGGCCTAC
VX10801: TTTTTTTTTTTTTTTGAATAACTTGAGG